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Water and Heat Balance during Flight in the Rose-Colored Starling (*Sturnus roseus*)

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ABSTRACT

Water imbalance during flight is considered to be a potentially limiting factor for flight ranges in migrating birds, but empirical data are scarce. We studied flights under controlled ambient conditions with rose-colored starlings in a wind tunnel. In one experiment, we measured water fluxes with stable isotopes at a range of flight speeds (9–14 m s⁻¹) at constant temperature (15°C). In a second experiment, we measured evaporation rates at variable ambient temperatures (T_a = 5°–27°C) but constant speed (12 m s⁻¹). During all flights, the birds experienced a net water loss. On average, water influx was 0.98 g h⁻¹ (SD = 0.16; n = 8), and water efflux was 1.29 g h⁻¹ (SD = 0.14; n = 8), irrespective of flight speed. Evaporation was related to temperature in a biphasic pattern. At temperatures below 18.2°C, net evaporation was constant at 0.36 g h⁻¹ (SD = 0.18; n = 10), rising at higher temperatures with a slope of 0.11 per degree to about 1.5 g h⁻¹ at 27°C. We calculated the relative proportion of dry and evaporative heat loss during flight. Evaporative heat loss at T_a < 18.2°C was 14% of total heat production during flight, and dry heat loss accounted for 84%. At higher temperatures, evaporative heat loss increased linearly with T_a to about 25% at 27°C. Our data suggest that for prolonged flights, rose-colored starlings should adopt behavioral water-saving strategies and that they cannot complete their annual migration without stopovers to replenish their water reserves.

Introduction

Flying birds have among the highest sustained metabolic rates known (Schmidt-Nielsen 1972; Norberg 1996; Butler and Bishop 2000). Only a minor part of metabolism is converted into mechanical work, whereas most of the energy ends up as heat (Hill 1938; Masman and Klaassen 1987; Biewener et al. 1992; Butler and Bishop 2000). A small amount of the excess heat can be stored by allowing body temperature to increase, as has been reported for resting birds under thermal stress (Dawson 1984; Withers and Williams 1990; Tieleman and Williams 1999) as well as for flying birds (Torre-Bueno 1976; Hudson and Bernstein 1981; Hirth et al. 1987; Adams et al. 1999). Given the high metabolic rates during flight, hyperthermia can buffer only a small proportion of the excess heat (Pearson 1964; Hart and Roy 1967; Craig and Larochelle 1991), and heat dissipation by dry heat transfer (convection, conduction, radiation) or evaporation is therefore of utmost importance for thermoregulation. At low ambient temperatures, that is, at a high temperature gradient between the bird and the surrounding air, dry heat transfer is very effective (Ward et al. 1999), but the conditions for dry heat transfer become increasingly unfavorable as ambient temperature rises. Birds can increase dry heat transfer behaviorally by decreasing their insulation and exposing well-vascularized body regions to the cooler environment. For example, flying birds may expose their feet to the airstream to increase the area of heat dissipation (Biesel and Nachtigall 1987; Ward et al. 1999; Kvist 2001, chap. 9; this study). At higher ambient temperatures, birds rely increasingly on evaporative cooling to maintain their body temperature at the expense of losing body water (Berger et al. 1971; Torre-Bueno 1978; Hudson and Bernstein 1981; Biesel and Nachtigall 1987; Adams et al. 1997; Dawson and Whittow 2000). During flight, they may open their beak to a variable degree or duration, exposing the moist inner side of the beak and thus promoting both evaporation and convection, a behavior that has been observed repeatedly during wind tunnel flights at higher temperatures (Biesel and Nachtigall 1987; St-Laurent and Larochelle 1994; Ward et al. 1999; Kvist 2001, chap. 9; this study).

We address the rate of water flux at variable wind speeds and ambient temperatures in the rose-colored starling (*Sturnus roseus*, Linnaeus), a long-distance migrant. In a bird's water budget, metabolic water production is the only appreciable source of water gain during flight. Metabolic water production depends directly on the metabolic rate and the substrate that is metabolized (Jenni and Jenni-Eiermann 1998; Willmer et al.

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2000). The influence of ambient temperature on flight costs, and thus heat production and metabolic water production, is assumed to be negligible in a moderate range of temperatures (Hudson and Bernstein 1981; Rothe et al. 1987). We therefore expect water influx to depend mainly on flight velocity (Pennycuik 1989; Rayner 1999). We measured water influx (metabolic water production plus possible uptake of humidity from the air) and efflux (total water loss) as well as the energetic costs of flight and heat production in relation to flight speed with doubly labeled water (DLW).

Evaporative water loss, in contrast, should be directly linked to ambient temperature and the water vapor pressure deficit (VPD; hPa) between the evaporating surfaces (skin, eyes, and respiratory tract) and the surrounding air (Gates 1980). We calculated evaporation rates from mass loss rates during flights at constant velocity but different ambient temperatures. From the evaporation rates we calculated evaporative cooling and the relative importance of dry heat transfer in flying rose-colored starlings.

In an attempt to pinpoint relevant physiological and meteorological factors that limit a bird's flight range, Carmi et al. (1992) and later Klaassen (1995) developed simulation models that predict that water imbalance rather than energy depletion may limit maximum flight duration under certain ambient conditions, namely, high ambient temperature and high flight altitude. Our data allow a tentative calculation of the maximum flight duration of rose-colored starlings to assess whether migration distance is limited by fuel or water depletion.

Material and Methods

Wind Tunnel

All experimental flights were conducted in a closed-circuit wind tunnel situated in the Max Planck Institute for Ornithology in Seewiesen, Germany (680 m above sea level), which closely resembles the wind tunnel in Lund, Sweden, in design and technical performance data (Pennycuik et al. 1997). Wind speed and temperature can be controlled, and ambient conditions (air pressure, humidity, temperature, speed) are automatically recorded by a data monitoring system (ARGUS, Sorbus) with a sampling rate of 1 Hz. We display and record the "equivalent air speed" (Pennycuik et al. 1997), which takes into account changes in air density that determine the forces on the wings and body of a bird. This ensures that experimental conditions at a certain velocity are comparable for all birds. During this study, temperature was controlled either by a heat exchanger and a cooler installed in the airstream or, to achieve high temperatures, by electrical heating fans that were introduced into the airstream upstream of the test section.

A fine net prevented the birds from leaving the flight chamber upstream. It was made of braided nylon cord 0.25 mm in diameter, with a square mesh of 18 mm \times 18 mm. Downstream, ca. 1.5 m from the test section, there was a net made

of braided nylon cord 1.0 mm in diameter, with a mesh of 12 mm \times 12 mm in a wooden frame.

Birds, Housing, and Training

Rose-colored starlings (*Sturnus roseus*) have a breeding distribution from central Asia to the Balkans and their winter quarters in northern India. They are long-distance migrants and cover their migration route mostly during daytime flights. In the wild, rose-colored starlings feed mainly on insects and fruit, foraging mostly on the ground, where they hunt for grasshoppers and other prey (Hudde 1993). One of the most westerly regular breeding colonies is found on the Crimea peninsula (Ukraine). This population makes a flight to and from India of about 5,000 km twice a year, and the birds should also be capable of long periods of flight in a wind tunnel.

The experiments were performed with 14 rose-colored starlings, four of which were purchased in 1999 as nestlings from a breeder; the others were taken in June 2001 as nestlings from a breeding colony on the Crimea peninsula, Ukraine. All birds were hand-raised at our institute. During the experimental periods, the birds were housed in groups of three or four in aviaries (ca. 2 m \times 1 m \times 2 m) adjacent to the wind tunnel and were given standard food consisting of insects, heart, rusk, and egg, supplemented with minerals and vitamins (Aviconcept) ad lib., some mealworms, and fresh fruit. They had access to fresh water and the opportunity to bathe at any time.

The light schedule was varied to simulate natural conditions for the birds: during winter, they had the photoperiod of their wintering quarters in northern India (27.5°N), which then changed gradually to the natural light conditions of their breeding grounds (47.5°N) during summer and back again. This kept the birds in their annual rhythm and in migratory disposition during the experimental phase, indicated by prior fattening and an increased willingness to fly. All experimental flights were conducted during the migration period. The birds were in good condition and had intact flight feathers during the entire experimental phase.

Before the DLW experiments, we gave the birds intense training at varying flight speeds to achieve a flight duration of 6 h, necessary for good accuracy of the isotope analysis. The four adult birds had been flying in the wind tunnel before and were ready for the experiments after 3 wk of regular training. The juvenile birds were given regular training flights for 8 wk before the experiments. During the last 2 wk before the experiments, all birds were trained daily for periods of 10–120 min at variable wind speeds, so that a total of 6 h of training per week was achieved.

The range of flight speeds that the birds could sustain for prolonged periods of flight was from 9 to 14 m s⁻¹. Attempts to train the birds at lower or higher velocities failed because after a short flight duration of usually only a few minutes, the birds landed frequently and eventually refused to start flying

again. At flight speeds above 14 m s^{-1} , the birds seemed to struggle against the wind and were sometimes blown against the rear net.

Our birds flew more steadily and with fewer attempts to land when they were led into the wind tunnel in groups. We therefore let them fly in pairs or in groups of three during both training and the experiments. The birds changed their position within the group frequently, and there was no indication that they used any particular flight formation that might save energy. None of the birds would fly systematically close to the floor or ceiling of the wind tunnel. Therefore, boundary layer effects (Rayner 1994) are unlikely.

Experimental Flights

Experiments were conducted in two series. In experiment 1, flights were conducted at a constant ambient temperature (15°C) but different flight speeds ($9\text{--}14 \text{ m s}^{-1}$) and lasted 6 h each. In this set, energetic costs of flight (EE_f ; W), total body water content before and after the flight (TBW_i and TBW_f , respectively; g), and water fluxes (rH_2O_{in} and rH_2O_{out} ; g h^{-1}) were measured with stable isotopes. In experiment 2, flights lasted 1 h each. Flight speed was held constant (12 m s^{-1}), but ambient temperature differed between flights ($5^\circ\text{--}27^\circ\text{C}$). Evaporation rates were calculated from mass loss rates. Heat balance was calculated from the combined data of both experimental series.

Experiment 1. TBW_i , TBW_f , rH_2O_{in} , rH_2O_{out} , and EE_f were investigated using the DLW method (Lifson and McClintock 1966; Speakman 1997; Kvist et al. 2001). Eight birds, four adults and four juveniles, flew repeatedly (two to six times) for 6 h at different wind speeds during the spring and autumn migration periods in 2001. Ambient temperature was 15.04°C ($SD = 0.09$; $n = 27$). The standard deviation within a flight never exceeded 0.54°C . Air pressure and humidity could not be controlled. Mean air pressure during the experiments ranged from 919.0 to 957.5 hPa, with an average of 938.8 hPa, and relative humidity was 60.65%, on average, ranging from 48.4% to 76.8%. This corresponds to a water vapor pressure of 10.4 hPa ($SD = 1.8$; $n = 27$). The VPD, the difference between the maximum and the actual vapor pressure at a given temperature, was 6.7 hPa ($SD = 1.8$; $n = 27$), on average, ranging from 3.9 to 8.7 hPa.

Wind speed changed between flights and ranged from 9 to 14 m s^{-1} , with an intraexperiment standard deviation (calculated over all measurements taken at 1-Hz sampling rate) of less than 0.12 m s^{-1} . The succession of wind speeds was chosen randomly. Individuals had no more than one experimental flight per week. Only flights with an apparently natural flight attitude were included in the analysis. In total, 27 flights were performed successfully.

Usually, the birds had been food deprived since the evening

before to have them postabsorptive during the flights. In eight cases, flights were conducted without the removal of the food the evening before. The birds then had access to the remaining food from first light until the beginning of the experiment at 7:00 a.m., an episode lasting between 60 and 90 min. Even if the birds had been feeding during this time, it would have had only a minor effect on the accuracy of the energy and TBW measurements (Speakman 1997).

Body mass was taken directly before and after the flight to the nearest 0.01 g. Flight behavior was continuously recorded by an observer for all flights, who also prevented the experimental birds from landing or from leaving the wind tunnel. In most cases, the mere attendance of the observer was sufficient for this. Sometimes, however, a bird decided to land frequently within a short period of time, in which case the observer waved at the bird to prevent it from landing. If an individual showed signs of exhaustion, it was given a rest or was taken out of the experiment. We took it as an indicator of exhaustion if a bird landed frequently, even when being chased up immediately after landing, if a bird ignored the observer and refused to start flying again, or if a bird was flying close to the exit of the wind tunnel, trying to escape. A resting episode occurred in four flights and lasted between 15 and 33 min.

Before each flight, an initial blood sample (sample 0) was taken for the determination of the background concentration of the isotopes in the bird. The bird was then injected intraperitoneally with 0.2 g of a DLW mixture of known isotope concentration (^2H enrichment was 30 atom percent, and ^{18}O enrichment was 60 atom percent). The quantity was assessed to the nearest 0.0001 g by weighing the syringe before and after the injection on an analytical balance (Sartorius BP121S). An equilibration period of 1 h without access to food or water allowed the isotopes to mix completely with the bird's body water before the next blood sample (sample 1) was taken and the bird was released into the wind tunnel for the flight. This blood sample allowed the calculation of the initial body water (TBW_i). After 6 h of flight, another blood sample (sample 2) was taken, and the bird was reinjected with 0.1 g of DLW (the exact quantity assessed gravimetrically, as described above). After an equilibration of 1 h, again without access to food and water, a last blood sample (sample 3) was taken that allowed the calculation of the final body water (TBW_f).

The blood samples were taken from the jugular vein to avoid hematoma at the brachial vein, which could disturb the bird during flight. Per sampling period, a total of about $75 \mu\text{L}$ of blood was taken, subdivided into four or five microcapillary tubes. All blood samples were flame sealed immediately and stored at 5°C for isotope analysis at the Centre for Isotope Research in Groningen, Netherlands. The samples were analyzed blindly, without knowledge of the flight conditions, and in quadruplicate to give a high degree of accuracy. For details of analysis, see Visser et al. (2000) and Kvist et al. (2001).

Total body water. The size of the body water pool at the start

and the end of each experimental flight was calculated by the principle of hydrogen dilution. Because the quantities of the DLW injections and the ^2H concentrations of the doses, as well as the ^2H concentration in the bird's body water pool before and after the DLW administrations, are known, we can calculate

$$\begin{aligned}\text{TBW}_i &= 18.02Q_{d1}(C_{d1} - C_i)/(C_i - C_b), \\ \text{TBW}_f &= 18.02Q_{d2}(C_{d2} - C_f)/(C_f - C_r),\end{aligned}\quad (1)$$

where TBW_i (g) is the initial size of the body water pool before a flight, TBW_f (g) is the final size of the body water pool after the completion of a flight, Q_{d1} and Q_{d2} (mol) are the quantities of the first and second DLW doses, respectively, C_{d1} and C_{d2} (atom percent) are the ^2H concentrations of the first and second doses, respectively, C_b (atom percent), determined from sample 0, is the ^2H concentration in the bird's body water pool before the initial DLW administration, C_r (atom percent), determined from sample 2, is the ^2H concentration in the bird's body water pool before the DLW reinjection, C_i (atom percent) is the ^2H concentration after the initial administration, measured in sample 1, and C_p (atom percent) is the ^2H concentration after the second administration, measured in sample 3. This method has been referred to as the plateau method (Speakman 1997; Visser et al. 2000).

Water flux rates. Because none of the animals were in a steady state with respect to body mass (i.e., body water), water efflux rates, consisting of evaporative and excretory water losses (uncorrected for fractionation effects; $r\text{H}_2\text{O}_{\text{unc}}$; g h^{-1}), were first calculated following Nagy and Costa (1980), who provided an equation that takes changes in the size of the water pool into account:

$$\begin{aligned}r\text{H}_2\text{O}_{\text{unc}} &= t(\text{TBW}_f - \text{TBW}_i) \\ &\times \frac{\ln \{[(C_i - C_b)\text{TBW}_i]/[(C_r - C_b)\text{TBW}_f]\}}{\ln (\text{TBW}_f/\text{TBW}_i)},\end{aligned}\quad (2)$$

where t is time expressed in hours. Second, a correction was made for isotope fractionation effects due to evaporative water loss that took into account a proportionally higher evaporation of nonlabeled molecules compared to heavy isotopes. Corrected water efflux rates ($r\text{H}_2\text{O}_{\text{out}}$; g h^{-1}) are calculated as

$$r\text{H}_2\text{O}_{\text{out}} = r\text{H}_2\text{O}_{\text{unc}}/(xf_1 + 1 - x) \quad (3)$$

(Speakman 1997), where x represents the proportion of the water flux lost through evaporative pathways (taken as 0.5, as proposed by Lifson and McClintock [1966]) and f_1 is the fractionation factor (taken as 0.94, as recommended by Speakman [1997, p. 107]). To assess the effect of the assumed fraction of evaporative water loss on the water flux rates, we made a comparison between the results obtained assuming values of fractional evaporative water loss of 0.5 and 1 (i.e., evaporation

accounts for 50% or 100% of the total water loss). Under the assumption that 100% of water is lost on evaporative pathways, $r\text{H}_2\text{O}_{\text{out}}$ was 3% higher than under the 50% assumption. The value of 1 is unlikely because some water will be lost by excretion. Giladi and Pinshow (1999) found that excretion accounted for about 10% of total water loss in flying pigeons. Therefore, we assume the maximum uncertainty in our water flux rates to be on the order of 2%.

Water influx rates ($r\text{H}_2\text{O}_{\text{in}}$; g h^{-1}) were calculated using Equation (3) and the fractionation-adjusted water efflux rates

$$r\text{H}_2\text{O}_{\text{in}} = r\text{H}_2\text{O}_{\text{out}} + (\text{TBW}_f - \text{TBW}_i)/t; \quad (4)$$

again, t is time expressed in hours. Here $r\text{H}_2\text{O}_{\text{in}}$ represents metabolic water produced from the combustion of substrate and an unknown fraction of the humidity in the inhaled air that might have been taken up in the respiratory tract.

Energy expenditure. EE_f was analyzed from isotope turnover rates between samples 1 and 2. The calculated metabolic rate was corrected for resting and handling phases by subtracting the energy expenditure of these nonflight periods, calculated as the resting metabolism during the active phase, according to Aschoff and Pohl (1970). Nonflight phases had a mean duration of 0.36 h (range: 0.20–1.07 h) and accounted for less than 0.5% of the total energy expenditure. The corrected value represents EE_p , which is taken for the calculation of gas exchange and metabolic water production later. A detailed analysis of the flight costs in relation to flight velocity is presented by Engel et al. (2006a).

Experiment 2. In the second series of flights, evaporation rates (total evaporative water loss [TEWL] and net evaporative water loss [NEWL]; g h^{-1}) were calculated from mass loss rates of a total of 58 flights of 1-h duration by 10 individuals. These flights were conducted by the same four adult birds that had been used during the DLW flights and by six juveniles. The adults flew during the migration periods autumn 2000, spring 2001, and autumn 2001, and the juveniles flew during the autumn migration period in 2001. An individual never had more than two experimental flights per week. The number of successful flights per bird was six, on average, and varied between two and 14.

Wind speed was kept constant during all flights at 11.98 m s^{-1} (SD = 0.06; $n = 58$), while ambient temperature changed between flights and ranged from 5.1° to 27.3°C , with SD < 0.58°C within a single flight. The succession of ambient temperatures was chosen randomly. Air pressure and humidity could not be controlled. Mean air pressure was 938.64 hPa (SD = 8.32; $n = 58$), and relative humidity was 51.53% (SD = 13.24; $n = 58$) for all experimental flights, corresponding to an average water vapor pressure of 8.7 hPa (SD = 2.1; $n = 58$). The VPD is the difference between the actual and the maximum vapor pressure at a given temperature and deter-

mines the driving force on evaporation. On average, VPD was 8.7 hPa (SD = 5.7; $n = 58$), ranging from 1.6 to 23.4 hPa.

The birds had been food deprived since the evening before the experiment to have them postabsorptive and also to reduce excretion during flight. Flights started between 8:00 a.m. and 10:00 a.m. and lasted 60 min (SD = 3.34; $n = 58$). Before a flight, the cloaca of the birds was covered with a small piece of cotton and adhesive tape to prevent excretory mass loss. These cloacal covers added less than 0.3 g to the bird's body mass and disappeared completely under the feathers, so that they did not increase the bird's drag in the airstream. The body mass of the birds, including the attached cloacal cover, was measured directly before and after the flight to the nearest 0.01 g. Measurements were included in the analysis only if the tape was dry at the outer surface and still well attached to the bird's body during the second weighing. This ensured that mass loss consisted only of evaporation and the combustion of fuel. Total mass loss (m_t ; g h⁻¹) can be described as

$$m_t = m_{ex} + TEWL - mH_2O_{catabol} + mCO_2 - mO_2, \quad (5)$$

where m_{ex} is the excretory mass loss rate, TEWL is the total evaporation rate, $mH_2O_{catabol}$ is water influx from metabolic water production and the liberation of structurally bound water during catabolism of body tissue, mCO_2 is the carbon dioxide production, and mO_2 is the mass gain rate through oxygen uptake (all components are in g h⁻¹). Since excretory mass loss was prevented during the experimental flights, m_{ex} is 0.

Catabolic water influx ($mH_2O_{catabol}$) and the mass loss rate from carbon dioxide production and oxygen consumption ($mCO_2 - mO_2$) depend on the metabolic rate and the composition of fuel that is combusted. The metabolic rate of rose-colored starlings during flight (EE_f) was derived from the previously described DLW measurements. Since EE_f did not change with flight speed (Engel et al. 2006a), we took the average flight cost of all individuals during 27 flights, which was 8.17 ± 0.64 W ($n = 8$). From this value, we assume 4% to be derived from protein breakdown and the rest from fat, which is in the range of empirical estimates for migrating birds (Jenni and Jenni-Eiermann 1998; Klaassen et al. 2000; Battley et al. 2001). Carbohydrates may contribute to the fuel composition at the onset of a flight but are rapidly replaced by fat and protein metabolism. Previous fasting entails an even quicker transition (Rothe et al. 1987; Walsberg and Wolf 1995). We therefore assume that carbohydrate combustion is negligible over the course of a 1-h flight. For the assumed fuel composition, we calculated an energy equivalent of 27.8 kJ L⁻¹ CO₂, according to Schmidt-Nielsen (1997), which allows us to calculate mCO_2 (g h⁻¹) and subsequently mO_2 from EE_f following the equation

$$mCO_2 = (MCO_2/MO_2) \times RQ \times mO_2, \quad (6)$$

where MCO_2 , the molecular mass of carbon dioxide, is 44.01 g mol⁻¹, and MO_2 , the molecular mass of oxygen, is 32.00 g mol⁻¹. The respiratory quotient (RQ) depends on the fuel that is metabolized and is 0.71 for the assumed fuel composition (Schmidt-Nielsen 1997). Catabolic water flux ($mH_2O_{catabol}$), that is, influx through metabolic water production and the liberation of structurally bound water, can be calculated from Table 1 of Jenni and Jenni-Eiermann (1998). The TEWL can now be calculated from Equation (5). The NEWL that a bird experiences is the difference between water influx (here taken as $mH_2O_{catabol}$) and total evaporation.

Heat Transfer. Heat production during flight is a function of energy expenditure. The high metabolic rates necessary during avian flight are only partly transformed into mechanical work (Butler and Bishop 2000); the rest is converted into heat and has to be dissipated to maintain a constant body temperature. Heat transfer during flight at ambient temperatures below body temperature T_{body} , as in our experiments, is always in the direction from the bird's body to the surrounding air. It can take place as evaporative heat loss or dry heat loss (mainly via convection and radiation).

Evaporative heat loss. Evaporative heat loss (H_e ; W) is the rate of evaporation (TEWL) multiplied by the heat of vaporization of water (q ; J g⁻¹):

$$H_e = qTEWL/3,600; \quad (7)$$

q varies slightly with the temperature of the evaporating surface. We use an approximation of $q = 2,443$ J g⁻¹ (Schmidt-Nielsen 1997).

Dry heat loss. Dry heat loss (H_d ; total and all components in W) is the total heat production (H_t) minus H_e and stored heat (H_s):

$$H_d = H_t - H_e - H_s. \quad (8)$$

Total heat production during flight is $H_t = P(1 - \eta)$, where P is the metabolic power as derived from the DLW measurements described above and η is the flight muscle efficiency that converts metabolic energy to mechanical energy. Estimates for η vary. Often values between 0.23 (Pennycuik 1989) and 0.18 (Ward et al. 2001) are taken, but there are even lower estimates of 0.10–0.16 (Chai and Dudley 1995; Kvist et al. 2001). Here we assume a flight muscle efficiency of 0.18.

Stored heat. H_s results in an increase of T_{body} and can account for only a minor fraction of the excess heat production, since T_{body} has to remain within physiological limits:

$$H_s = C \times m_b \times (T_{fly} - T_{rest})/\Delta t, \quad (9)$$

where C is the specific heat of body tissue, 3.5 J g⁻¹ °C⁻¹ (Dawson and Whittow 2000), m_b is body mass (g), T_{fly} is body

temperature in flight ($^{\circ}\text{C}$), T_{rest} is body temperature at rest ($^{\circ}\text{C}$), and Δt is the time spent during flight (h). We assume a T_{rest} of 41°C , reported for the European starling (*Sturnus vulgaris*; Bezzel and Prinzinger 1990), a close relative to the rose-colored starling of similar size. Steady state measurements of T_{fly} range from 42° to 45°C (Hart and Roy 1967; Aulie 1975; Hudson and Bernstein 1981; Hirth et al. 1987). For the European starling, Torre-Bueno (1976) measured T_{fly} of 42.7° and 44.0°C . Here we assume an increase of body temperature during flight by 2.3°C to T_{fly} of 43.3°C , the average of Torre-Bueno's measurements and slightly lower than the mean of 74 species reviewed by Prinzinger et al. (1991).

Statistical Analysis

Results are presented as means ± 1 standard deviation. Water flux rates ($r\text{H}_2\text{O}_{\text{in}}$, $r\text{H}_2\text{O}_{\text{out}}$) in relation to flight speed and evaporation rates (TEWL) in relation to ambient temperature were analyzed separately. Significance was accepted at $P \leq 0.05$ (two-tailed).

Water flux rates. We used the statistical package MlwiN (Multilevel Models Project; Institute of Education, London) to test for the effect of speed, VPD, and M on $r\text{H}_2\text{O}_{\text{in}}$ and $r\text{H}_2\text{O}_{\text{out}}$ with mixed-models analyses. These models allowed the analysis of repeated measurements on the same individual (e.g., at different wind speeds) and associated differences in variances. Nonsignificant parameters were excluded stepwise, except the manipulated variable, speed.

Mass loss rates. We applied a continuous two-phase linear regression model (Koops and Grossman 1993; Kwakkel et al. 1993) to describe the relationship of evaporation rate to ambient temperature and to determine the temperature at which the evaporation rates started to increase. The general model was

$$Y = \alpha + \beta_i X - [r(\beta_i - \beta_{i+1}) \ln(1 + e^{(x-y_i)/r})], \quad (10)$$

where Y is the dependent variable, X is the independent variable, α is the intercept, β is the slope, y_i is the estimated breakpoint between phases i and $i+1$, and r is a smoothness parameter that was set at 0.05 (Koops and Grossman 1993). All curves were fitted using the nonlinear regression algorithm procedures from the NONLIN package (shareware program, P. H. Sherrod). The significance of adding a second phase to the model was assessed by an F -test to verify the biphasic nature of the relationship (Kwakkel et al. 1993).

For further analysis, the data set was divided into two parts, one below the breakpoint and one above. These parts were analyzed separately with mixed models that tested for the effect of ambient temperature (T_a), VPD, and M on evaporation rates. Again, parameters that were least significant were excluded stepwise, except the manipulated variable, T_a .

Results

Experiment 1

TBW was measured in eight individuals before and after 27 flights of 6-h duration each at flight velocities ranging from 9 to 14 m s^{-1} . Average m_b before the flights was $71.6 \pm 9.9 \text{ g}$ ($n = 8$). Average TBW_i was $42.9 \pm 4.0 \text{ g}$ ($n = 8$), and TBW decreased significantly during the flight period, to an average TBW_f of $41.1 \pm 4.0 \text{ g}$ ($n = 8$; paired t -test: $t = 14.29$, $P < 0.001$). Water efflux ($r\text{H}_2\text{O}_{\text{out}}$), consisting of evaporative water loss and the wet part of excretion, was $1.29 \pm 0.14 \text{ g h}^{-1}$ ($n = 8$), on average, and water influx ($r\text{H}_2\text{O}_{\text{in}}$), which is metabolic water production and humidity from the inspired air that might have been absorbed in the respiratory tract, was $0.98 \pm 0.16 \text{ g h}^{-1}$ ($n = 8$). The difference between $r\text{H}_2\text{O}_{\text{in}}$ and $r\text{H}_2\text{O}_{\text{out}}$ is the net water loss that the birds experienced during flight (Fig. 1). The birds suffered a net water loss in all cases of $0.31 \pm 0.06 \text{ g h}^{-1}$ ($n = 8$), on average, ranging from 0.11 to 0.58 g h^{-1} ($n = 27$).

To test whether part of the variance in water flux rates could be explained by differences in body mass or other parameters, we conducted a mixed-models analysis, including initial body mass (m_{bi}), speed, EE_p , and VPD as predictors for $r\text{H}_2\text{O}_{\text{in}}$ and $r\text{H}_2\text{O}_{\text{out}}$. The levels of water efflux could not be explained by any of the parameters. Water influx was dependent only on m_b (coefficient = 0.013, SE = 0.003, $P < 0.002$). On average, EE_f was $8.17 \pm 0.64 \text{ W}$ ($n = 8$), and it did not change with speed but was dependent on m_b (Engel et al. 2006a).

Experiment 2

During 58 flights at T_a between 5.1° and 27.3°C , 10 birds with an average m_{bi} of $75.61 \pm 7.05 \text{ g}$ experienced a mass loss of

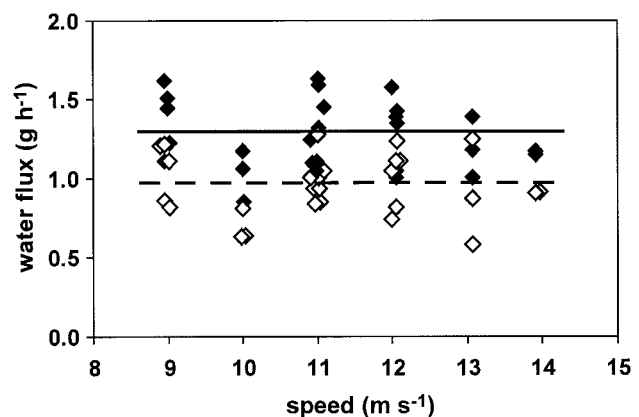


Figure 1. Water fluxes during flight in rose-colored starlings as measured with the doubly labeled water method. Open diamonds represent water influx (average value of 0.98 g h^{-1} ; dashed line); filled diamonds represent water efflux (average value of 1.29 g h^{-1} ; solid line). Flights lasted 6 h each and were performed at 15°C and at flight speeds ranging from 9 to 14 m s^{-1} .

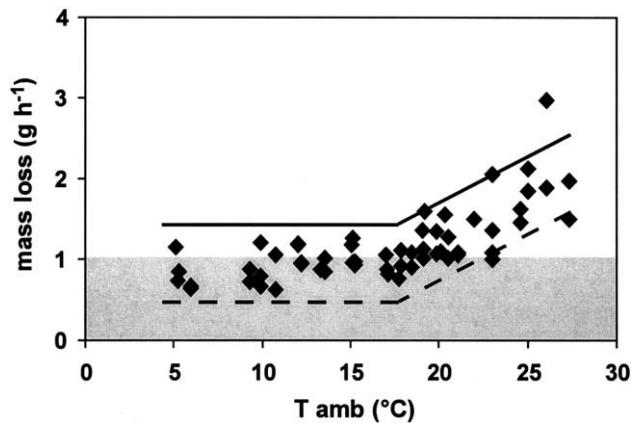


Figure 2. Rates of mass loss (diamonds), total evaporation (solid line), and net evaporation (dashed line) of rose-colored starlings in 1-h flights at 12 m s⁻¹ at ambient temperatures between 5° and 27 °C. A biphasic regression best described the relationship of total and net evaporation rates to ambient temperature. The gray bar represents catabolic water production, assuming flight costs of 8.17 W, as calculated from the doubly labeled water measurements in experiment 1, and a fuel composition of 96% fat and 4% protein. The birds were prevented from excreting by a cloacal cover.

1.17 ± 0.43 g h⁻¹ ($n = 10$), which corresponds to 2% of body weight per hour. With the subtraction of mass loss due to gas exchange and mass gain due to water influx, we calculated a TEWL of 1.63 ± 0.43 g h⁻¹, ranging from 1.09 to 3.42 g h⁻¹ (Fig. 2).

A two-phase regression described the relationship between TEWL and T_a significantly better than one linear regression ($F_{2,55} = 7.005$, $P < 0.02$). The breakpoint that divided the two phases was 18.2°C (SE = 1.33). Below this value, TEWL was temperature independent at 1.38 ± 0.18 g h⁻¹ (slope1 = 0.014, SE = 0.013, $P > 0.05$). At temperatures above 18.2°C, TEWL was 1.89 ± 0.47 g h⁻¹, on average, and increased with T_a (slope2 = 0.111, SE = 0.019, $P < 0.001$). The data set was therefore subdivided and tested separately for the effects of T_a , VPD, and m_{bi} . At $T_a < 18.2^\circ\text{C}$, there was no effect of T_a , VPD, or m_{bi} on evaporation rates. At $T_a > 18.2^\circ\text{C}$, there was a significant effect of VPD on TEWL (coefficient = 0.011, SE = 0.005, $P < 0.0001$).

We assume that water influx is not dependent on T_a in the range studied, because flight costs are supposed to be independent of temperature (Hudson and Bernstein 1981; Rothe et al. 1987). Under this assumption, the observed biphasic pattern also holds true for NEWL, which is total evaporation (TEWL) minus water influx, here catabolic water ($m\text{H}_2\text{O}_{\text{catabol}}$; g h⁻¹). The $r\text{H}_2\text{O}_{\text{catabol}}$ calculated from EE_f and the assumed fuel composition of 96% fat and 4% protein (Jenni and Jenni-Eiermann 1998; Klaassen et al. 2000; Battley et al. 2001), was 1.00 g h⁻¹, a value in close agreement with the water influx of 0.98 g h⁻¹, as determined for the DLW flights. The calculation

total evaporation minus water influx resulted in an average NEWL of 0.63 g h⁻¹. At $T_a < 18.2^\circ\text{C}$, NEWL was 0.38 ± 0.18 g h⁻¹, and at temperatures between 18° and 27°C, NEWL was 0.36–2.42 g h⁻¹.

Heat Balance

Heat production (H_t) during flight is a function of energy expenditure. With average EE_f of 8.17 ± 0.64 W, as measured in the DLW flights, and an assumed flight muscle efficiency of 0.18, H_t is 6.70 ± 0.52 W. An increase in body temperature during flight by 2.3°C results in 576 J of stored heat (H_s), corresponding to 0.16 W, or 2.4% of H_t , in 1 h. The remaining 6.54 W of heat production has to be dissipated by convection, radiation, or evaporation.

The quantities H_t and H_s are assumed to be constant over the range of T_a measured. But the relative contributions of heat loss avenues change with T_a (Fig. 3). At temperatures below the breakpoint of 18.2°C, TEWL = 1.38 g h⁻¹, on average. Since the evaporation of 1 g of water requires 2,443 J (Schmidt-

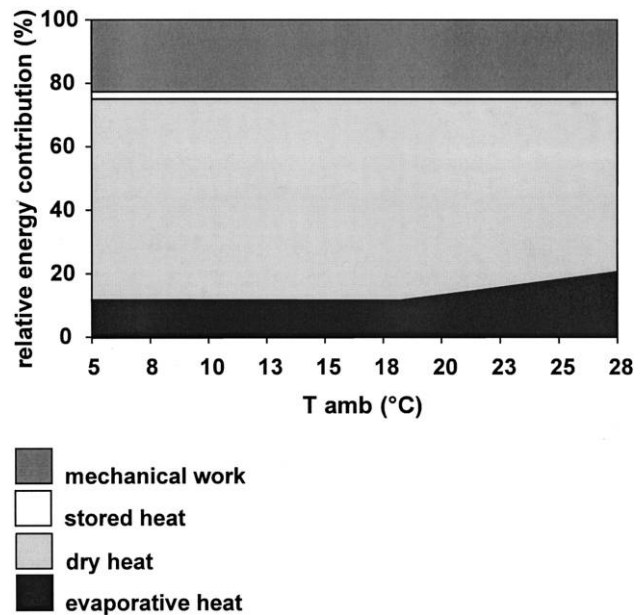


Figure 3. Heat balance model for a flying rose-colored starling, assuming flight costs of 8.2 W (taken as 100% of energy expenditure), as measured with the doubly labeled water method during flights of 6-h duration at 12 m s⁻¹ and 15°C. Flight muscle efficiency is assumed to be 18%, resulting in mechanical work of 1.5 W, the rest (82% of energy expenditure) being heat production (H_t). Hyperthermia of 2.3°C (a value taken from literature) results in heat storage of 0.16 W, or 2.4% of heat production during 1 h. Evaporation rates are calculated from mass loss rates during flights of 1-h duration at 12 m s⁻¹ and ambient temperatures (T_a) between 5° and 27°C and account for evaporative heat dissipation of 0.9–1.7 W, or 14%–25% of H_t , depending on T_a . Dry heat transfer is calculated as the difference between H_t and evaporative heat loss.

Nielsen 1997), this evaporation rate results in a heat loss (H_e) of 0.94 W, which is 14.0% of H_t ; the rest (5.60 W, or 83.6%) has to be dissipated by dry heat transfer (H_d). At higher T_a , evaporation rates increase linearly, resulting in a higher proportion of H_e relative to H_d . At 27°C, the highest temperature that was measured, 2.5 g of water are evaporated per hour, increasing H_e to 1.70 W, or 25.4% of heat production.

Discussion

Our measurements of water fluxes and evaporation rates in flying rose-colored starlings revealed that the birds had a negative water balance during all flights. Water efflux during the DLW measurements was 1.29 g h⁻¹, on average, standing against a water influx through metabolic water production and humidity from the respiratory air of 0.98 g h⁻¹. The resulting net water loss of 0.31 g h⁻¹ is statistically not different from the calculated NEWL of 0.38 g h⁻¹ at $T_a < 18^\circ\text{C}$, derived from the mass loss measurements ($t = 1.66$, $df = 11$, $P > 0.05$).

Evaporation followed a biphasic pattern, with relatively low and constant rates at the lower range of temperatures and an abrupt increase at T_a above 18.2°C. A biphasic evaporation pattern has also been reported for other birds during flight (Tucker 1968; Torre-Bueno 1978; Giladi and Pinshow 1999; Kvist 2001, chap. 9). European starlings (Torre-Bueno 1978) experienced a TEWL of 1.1 g h⁻¹ at 5°C, this rate being relatively constant throughout a temperature range up to about 15°C. At higher temperatures, evaporation rates rose to reach 3.0 g h⁻¹ at 29°C. Our results are in good agreement with these measurements. Some authors (Hudson and Bernstein 1981; Biesel and Nachtigall 1987) found constant increases of evaporative water loss with T_a (minimum T_a of 18° and 7.5°C, respectively). While the measurements in white-necked ravens (Hudson and Bernstein 1981) may represent only the temperature range above the threshold temperature, the linear increase in evaporation rates in pigeons (Biesel and Nachtigall 1987) is less easily explained, particularly given other measurements in pigeons (Giladi and Pinshow 1999) that revealed the expected biphasic pattern with a threshold temperature between 15° and 19°C. Maybe evaporative cooling had to be initiated at a lower temperature in the former wind tunnel study compared to the latter free-flight measurements because of increased metabolic demands (Giladi and Pinshow 1999).

Thermal imaging led to the calculation of a heat balance model for flying European starlings (Ward et al. 2004). This approach allows the measurement of radiative heat transfer and the calculation of convection and metabolic power in unrestrained birds. Convection was the most important mechanism for heat transfer in that study, accounting for almost 80% of total heat transfer, while radiation accounted for only 9%. During these measurements, dry heat transfer, the sum of radiation and convection, was 8.9 W at 12 m s⁻¹ (the flight speed of our mass loss measurements). This value is somewhat higher than

our calculated value for the rose-colored starling (5.6 W). Several aspects may contribute to this difference. (1) Rose-colored starlings may generally differ from European starlings. To assess between-species differences, we certainly need more data for comparison. (2) Body mass differences may account for different flight costs, which are the basis for our model calculation. Our birds had an average body mass of 71.6 g during the DLW measurements, compared to 82.0 g for the European starlings. Accordingly, metabolic flight costs differed (8.2 W in rose-colored starlings, measured with DLW, vs. 11.6 W in European starlings, calculated from heat transfer modeling). (3) There may be differences in the underlying model assumptions. Our calculations are based on flight metabolic rate (measured during DLW flights), evaporation rate (calculated via mass loss rates), an assumed efficiency for the conversion of metabolic power to mechanical power of 18%, and an assumed increase in T_b of 2.3°C. Errors in both assumptions have a direct effect on the resulting value for dry heat transfer. If we assume an efficiency of 15%, comparable to the calculations of Ward et al. (2004), H_t would increase to 6.9 W. Furthermore, we assume hyperthermia during flight and thereby heat storage of 0.2 W, while Ward et al. (2004) assumed that body temperature remains constant (which would increase our H_t further to 7.1 W). Despite the differences discussed above, when expressed in relative terms, the two models result in very similar proportions of dry heat transfer compared to total heat flow (88.5% in European starlings and 82.5% in rose-colored starlings).

To assess the sensitivity of our results to the underlying assumption of fuel composition (96% of energy derived from fat, 4% from protein), we also calculated evaporation rates from our mass loss data, under the assumptions that (1) only fat is used to fuel flight and (2) 10% of the energy is derived from protein breakdown. This increase in the proportion of protein catabolism results in an increase of RQ by 0.4%. The $m\text{CO}_2$ increases by 2%, as does the $m\text{O}_2$. NEWL decreases by 3%, from 0.64 to 0.62 g h⁻¹. In contrast to these small changes stands a huge increase in water gain, from 0.85 to 1.22 g h⁻¹ with increasing protein use, that is mainly due to the high water content of wet protein (Jenni and Jenni-Eiermann 1998) liberated when tissue is catabolized. Accordingly, TEWL increases by 24%, from 1.49 to 1.84 g h⁻¹. While NEWL, and therefore water balance during flight, is relatively insensitive to errors in the assumed fuel composition, total evaporation changes dramatically, making heat balance calculations much less reliable. The actual error introduced by our assumed fuel composition is probably much smaller than in these extreme cases, as the good fit between DLW measurements and mass loss calculations suggests. However, more information on fuel composition during bird flight would be of great value to future modeling.

Our birds experienced a minimum evaporation during flight that exceeds water gain through the catabolism of body stores and possibly respiratory air, resulting in a net water loss at all measured T_a . A negative water balance during flight imposes a

potential limit to flight duration through water depletion, as has already been proposed on the basis of theoretical considerations (Carmi and Pinshow 1995; Klaassen 1995, 2004; Klaassen et al. 1999). If water imbalance is a real problem, as our measurements suggest in accordance with previous studies, a bird during migration should seek to minimize water loss behaviorally and physiologically. Possible behavioral mechanisms are the choice of favorable weather conditions, especially with respect to T_a , which includes the choice of flight altitude or possibly a night migration strategy. Physiological mechanisms might target excretory water loss and cutaneous and respiratory evaporation. The excretion of metabolic waste products in the form of uric acid might be an example of physiological adaptation to water economy. Measurements in free-flying pigeons show that excretory water loss still is a substantial proportion (almost 10%) of total water loss, at least in this species (Giladi and Pinshow 1999). Cutaneous evaporation has long been thought to be of minor importance for avian water balance because birds have no sweat glands (Rawles 1960; Bartholomew and Cade 1963), but later work has shown that cutaneous evaporation is an important avenue of water loss in the thermoregulatory process. Most of the available studies were conducted with resting birds (Bernstein 1969; Dawson 1982; Marder and Ben-Asher 1983; Webster and Bernstein 1987; Webster and King 1987; Wolf and Walsberg 1996; Tieleman and Williams 2002), but at least for pigeons, cutaneous evaporation has been shown to be an important avenue of water loss during flight as well (Michaeli and Pinshow 2001). Given the high respiration rates during flight (Berger et al. 1970; Butler 1980; Bernstein 1987), the relative contribution of respiratory evaporation to total evaporation is likely to be high and might set a lower limit to evaporation rates. Respiration is a vital function underlying other and stronger needs than the need for water saving. It might not, therefore, be easily accessible to the evolution of water-saving mechanisms. Measurements on flying pigeons (Michaeli and Pinshow 2001) and ducks (Engel et al. 2006b) suggest that there is a certain water-saving potential in the cooling of exhaled air below body temperature at low to intermediate T_a . The underlying physiological mechanisms remain to be investigated.

We can use our measurements of EE_p , m_b , TBW, and evaporation rates to calculate maximum flight duration for our rose-colored starlings and then to determine whether flight duration is more likely limited by energy or by water depletion (Biebach 1991; Carmi et al. 1992; Klaassen 1995; Leberg et al. 1996; Klaassen et al. 1999). First, we can estimate body composition of our experimental birds. Given the average m_b (71.6 g) and TBW_i (42.9 g) and assuming that the TBW : lean dry mass is 0.7 : 0.3 (Ellis and Jehl 1991), we calculate a lean dry mass of 18.4 g and a fat content of 10.3 g. Given that the energy content of fat is 39.3 kJ g⁻¹ (Schmidt-Nielsen 1997), our measured flight costs of 8.2 W (Engel et al. 2006a) would allow our starlings to fly for 13.7 h if they use only their 10.3 g of stored fat for

fuel. This is a conservative estimate, because mass loss during this time period will reduce flight costs and thus increase maximum flight duration. For example, our DLW measurements showed that in rose-colored starlings, flight costs scale with body mass to the power of 0.554 (Engel et al. 2006a). Dehydration at the minimum evaporation rate of 0.38 g h⁻¹ would reduce the bird's mass by 5.2 g over 13.7 h and thereby increase the possible flight duration to 14.2 h. On the other hand, fat has the highest energy density of all tissues (Jenni and Jenni-Eiermann 1998); any combination with other substrates will result in a shorter flight duration.

To what extent flight distance is limited by water depletion depends on the rate of net water loss and the tolerable degree of dehydration during flight. Estimates of the maximum endurable water loss in flying birds lie in the range of 15% of body mass (Berger and Hart 1974; Carmi et al. 1993) or 30% of TBW_i (Carmi et al. 1992), corresponding to an allowable net water loss of 11–13 g in rose-colored starlings. The rate of NEWL depends on T_a : at temperatures below 18.2°C, we calculated an average net water loss of 0.39 g h⁻¹, which would allow a flight duration of 28.9 h if we assume a maximum allowable water loss of 11 g. In this case, flight duration is clearly limited by fat depletion if rose-colored starlings have only 10.3 g stored fat, as in our captive birds. Flights at 27°C, however, were associated with a net water loss of up to 2.42 g h⁻¹, reducing the potential flight duration to 4.5 h. The critical

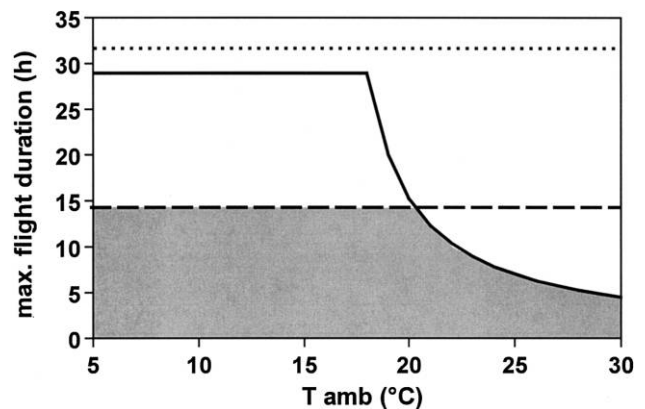


Figure 4. Estimates of maximum flight duration for our experimental birds based on fat depletion or dehydration, assuming that flight costs are independent of ambient temperature (T_a) and that maximum tolerable dehydration is 15% of body mass. Body composition was estimated as described in the text. Ten grams of fat can fuel flight for 14 h (dashed line). In contrast, dehydration (solid line) would limit flight duration to 29 h at $T_a < 18^\circ\text{C}$. At $T_a > 18^\circ\text{C}$, evaporation increases rapidly, and maximum tolerable dehydration would occur after only 5 h at 27°C . Maximum flight duration of a bird (gray area) is limited by fat depletion at $T_a < 21^\circ\text{C}$ and by dehydration at $T_a > 21^\circ\text{C}$. Note that changes in body composition change the model outcome considerably: 50 g of fat could fuel flight for 32 h (dotted line). In this case, flight duration would be limited by dehydration at all temperatures.

temperature above which water becomes the limiting factor for flight duration, compared to the estimates for fuel depletion, is about 21°C (Fig. 4). We know little about the actual body composition of rose-colored starlings in the wild. An early study reports that body mass was 50–60 g upon arrival in the wintering quarters and 80–125 g before the onset of migration (Naik 1963). Most of this mass increase must be due to the accumulation of fat. While TBW, and therefore the maximum allowable water loss, changes little with increasing fat load, energy stores of 50–60 g fat at the start of migration could fuel flight for more than 30 h. Under these conditions, flight duration will be limited by dehydration even at minimum evaporation rates.

What are the actual ambient conditions that rose-colored starlings experience during their migratory flights? Rose-colored starlings are considered diurnal migrants. Several populations will have to cross Iran on the way to and from the wintering quarters in India; therefore, we chose average temperature conditions in Tehran as exemplary for the conditions during the migratory phase. Average daily temperatures are between 14° and 28°C in May, during spring migration, and 12°–24°C in October, during autumn migration (information from the BBC Weather Centre). Flight altitude has a slight effect on energy expenditure, because it is more costly to produce the necessary lift at low air density (high altitude). More pronounced, however, is the effect on water balance, because air temperature decreases with altitude, by roughly 6°C per 1,000 m elevation. Flying at high altitude and therefore low T_a would facilitate dry heat loss and reduce the need for evaporative cooling. At the same time, the lower oxygen partial pressure would increase the rate of respiratory water loss through increased lung ventilation. At an assumed flight altitude of 1,000 m above ground (Kerlinger 1995; Klaassen and Biebach 2000; Maybee and Cooper 2004), T_a will be reduced to an average of 8°–22°C in May and 6°–18°C in October. These temperature ranges seem favorable to maintaining minimal evaporation levels for most but not all of the time. There are no ecological barriers like high mountain ranges or oceans between wintering and breeding grounds of rose-colored starlings, and they can theoretically stop and replenish their body reserves at any time of the journey. Our findings suggest that they cannot complete their annual migration in one single nonstop flight but have to feed and replenish their water reserves during several stopovers.

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